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ADRENERGIC FACTORS IN GLUCOSE HOMEOSTASIS

CHARLES FREDERICK SCHOLHAMER, JR.

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ADRENERGIC FACTORS IN GLUCOSE HOMEOSTASIS

by

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B.A. Yale University, 1968

A thesis presented to the faculty of the Yale
University School of Medicine in partial
fulfillment of the requirement for the
degree of Doctor of Medicine

Yale University School of Medicine
Department of Internal Medicine
-April, 1972-

To my wife, Susan

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INTRODUCTION:

Since the initial work of Cori and Cori (1-5) on the effects of epinephrine on glucose homeostasis, many investigations have been undertaken to clarify the role of adrenergic factors in the economy of carbohydrate metabolism. In particular, epinephrine has been shown to stimulate liver and muscle glygogenolysis in vivo (4, 6, 7, 8) and in vitro (9, 10) resulting in increases in glucose and lactic acid concentrations in the blood. Also, epinephrine has been shown to stimulate lipolysis with the consequent release of free fatty acids and glycerol into the bloodstream (11, 12). In addition, infusions of epinephrine in diabetic children have been shown to greatly increase the level of ketones in the blood (13). More recently, the role of epinephrine vis-a-vis insulin secretion has been investigated with the demonstration that epinephrine inhibits insulin output (14).

Although the above mechanisms of epinephrine's action have been elucidated, little is known regarding the effect of catecholamines on amino acid metabolism and gluconeogenesis. Specifically, the effect of adrenergic factors, particularly, epinephrine, upon individual arterial amino acid levels and splanchnic uptake of amino acids has not been elucidated. Previous evidence suggests a decrease in the total amino acid content of the blood after epinephrine (15, 16, 17) but this does not take into account either the individual amino acid levels or the balance across the liver. With regard to the role of epinephrine in the splanchnic utilization of amino acids evidence is available only in terms of nonmetabolizable amino acids. Specifically, Chambers et. al.

found an increased uptake of the nonmetabolizable amino acid, α-aminoisobutyric acid (AIB), in the perfused rat liver after in vivo pretreatment with epinephrine (18). Sanders and Riggs reported an increased uptake of two nonmetabolizable labelled amino acids following insulin-induced hypoglycemia suggesting an effect of endogenous epinephrine in restoring glucose balance (19). However, in neither study were endogenous amino acid levels measured in arterial blood nor was splanchnic amino acid balance investigated. This lack of information assumes importance in view of the recent demonstration of the prominent role of endogenous amino acids, in particular alanine, in the regulation of gluconeogenesis (20-25).

With the delineation of the concept of α and β receptors at the tissue level by Alhquist in 1948 (26) much investigation has also gone into a description of the specific receptors at different tissue sites and of the effects of blocking agents in modifying the effects of adrenergic agents on splanchnic and peripheral glucose metabolism. In recent years the work of Porte and his colleagues has demonstrated the important role of the adrenergic nervous system in glucose and insulin homeostasis (12, 14, 27, 28). In particular, several recent reports indicate the propensity of propranolol, a β-adrenergic blocking agent, to cause hypoglycemia in the dog and man. Measuring venous glucose levels in anesthetized dogs, Berk and his co-workers described profound hypoglycemia after intravenous propranolol over a dose range of 25-600 μg/kg (29). Reports in man over the past few years confirm this hypoglycemic potential of propranolol and have demonstrated its ability to blunt glucose recovery after insulin-induced hypoglycemia. In 1966, Kotler and his group reported that propranolol precipitated severe attacks of hypoglycemia in a patient who

had previously undergone a partial gastrectomy and in an insulin-dependent, juvenile onset diabetic (30). In the same year, Abramson et. al. observed that propranolol dampened the glucose and free fatty acid rebound to insulin-induced hypoglycemia (31). In 1967, Mackintosh reported the case of a nine year old girl who suffered hypoglycemic episodes after intravenous propranolol during an ear operation and after a 24 hour fast (32). Again in 1967, Bewsher observed that oral propranolol lowered blood glucose in exercising subjects (33). However, in contrast to the above mentioned reports, Allison and his group in 1969 found that therapeutic oral doses of propranolol did not produce significant hypoglycemia in normal subjects even after a 24 hour fast or after walking up an inclined plane (34).

Despite these reports, the mechanism by which propranolol precipitates or potentiates hypoglycemia has not been clarified. The various hypotheses proposed have included blockade of hepatic glycogenolysis (29, 30); inhibition of muscle glycogenolysis and peripheral lipolysis with a concommitent decrease in substrate availability for gluconeogenesis (30, 31, 35, 36); an increase in peripheral glucose utilization (36); and possibly direct stimulation of insulin secretion (37). Lacking in all these reports has been a definite correlation between the observed hypoglycemia of propranolol and its effects upon simultaneously determined hepatic glucose production and peripheral glucose utilization.

The present study was designed to further delineate the effects of the adrenergic nervous system on glucose homeostasis. Using the hepatic venous catheter technique in anesthetized dogs two specific areas of investigation

have been pursued:

(1) A delineation of the effects of epinephrine infusion on arterial concentrations and splanchnic exchange of individual amino acids; and

(2) An examination of the relative effects of propranolol on splanchnic glucose production and peripheral glucose consumption.

In the presentation of the results and in the discussion below, these two areas of investigation are treated separately, under the headings "Epinephrine" and "Propranolol".

METHODS:

SUBJECTS

Twenty-one adult mongrel dogs ranging in weight from 18 to 26 kg. were maintained on an ad. lib. standard laboratory diet with free access to water. On the day of experimentation after a 12 to 18 hour fast the dogs were anesthetized with sodium pentobarbital (30 mg/kg) and placed on a Harvard respirator. In the propranolol study the animal's heart rates were continuously monitored by electrocardiography (limb leads).

CATHETERIZATION, SPLANCHNIC BLOOD FLOW, AND INFUSIONS

Polyethylene catheters were inserted into an exposed femoral artery, femoral vein, and external jugular vein, with an additional catheter placed distally in a femoral vein draining the hind limb in the propranolol study. A single lumen end-hole cardiac catheter was placed via the opposite external jugular vein under fluoroscopic control into the left hepatic vein (38-40). The catheter was placed mid-position between the entrance to the hepatic vein and the wedge position. The position of the hepatic venous catheter was checked continuously by fluoroscopy during the experiment. The catheters were kept patent by intermittent flushing with saline. No anticoagulants were used with the exception of four of the dogs in the epinephrine study who received heparin (5 mg/kg).

Splanchnic blood flow was estimated by the continuous infusion technique

of Bradley and Ingelfinger (41) employing indocyanine green dye* (42). After the catheters were in place a priming dose of indocyanine green dye was given (0.3 to 0.5 mg/kg) followed by a constant infusion of the dye by a Harvard infusion pump via an external jugular venous catheter at rates of 0.081 to 0.134 mg/min (43). After a 15 minute equilibration period, simultaneous arterial and hepatic venous samples were obtained for blood flow analysis. In the epinephrine study samples were collected for blood flow determination every 10 minutes during a 30 minute control period, and at 10-20 minute intervals for 100 minutes following initiation of the infusion. In the propranolol study samples were collected every 15 minutes during the 30 minute control period and for 180 minutes after initiation of the infusion.

Following a 30 minute control period, epinephrine† was infused in seven dogs over 60 minutes by means of a Harvard infusion pump via a femoral vein in a dose of 0.1 μg/kg/min. The epinephrine was diluted in a total volume of 50 ml. of isotonic saline to which was added 250 mg. of ascorbic acid as an antioxidant. Blood samples for chemical analysis were obtained simultaneously from the femoral artery and hepatic vein at 10 minute intervals during the control period, and at 10-20 minute intervals during the infusion and for 40 minutes following completion of the infusion.

Propranolol was administered to nine dogs following a 30 minute control period. The propranololψ was given in a dose of 150 μg/kg dissolved in

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* Supplied as Cardiogreen by Hynson, Westcott, and Dunning.

† Adrenalin Chloride, Parke-Davis.

ψ Inderal, Ayerst.

8 to 12 ml. of isotonic saline and injected intravenously over 3 minutes. Blood samples for chemical analysis were obtained from the arterial, hepatic venous, and deep femoral venous catheters at 30 minute intervals during the 30 minute control period and for 180 minutes following completion of the infusion. In an additional five dogs a 3 minute infusion of 8 to 12 ml. of isotonic saline was administered followed by blood sampling at the same intervals as in the group receiving propranolol.

COLLECTION, BLOOD ANALYSIS, AND STATISTICAL ANALYSIS

Blood samples were collected and placed in heparinized tubes at 4° C until the end of the study. At this time the heparinized samples were centrifuged at 4° C. Aliquots of the separated plasma were analyzed for indocyanine green dye or frozen at -19° C for future analysis of glucose, insulin, and amino acids as indicated. Blood glucose was determined in duplicate by the glucose oxidase method (44). Individual amino acids were determined in the epinephrine study by the automated ion-exchange chromatography technique (45) on heparinized plasma deproteinized with sulfosalicyclic acid (46). Plasma insulin was measured in the propranolol study by immunoassay using talc to separate bound and free insulin (47). The paired t-test was employed in both studies for statistical analysis (48).

RESULTS:

I. EPINEPHRINE

1. Basal Glucose Metabolism

During the control period, as seen in Figure 1 and Table I, the hepatic venous glucose levels consistently exceeded those of arterial blood indicating a net splanchnic release of glucose. Splanchnic glucose production, calculated from the estimated hepatic blood flow and the hepatic venous-femoral arterial glucose difference, and taken as a mean of the three sampling periods, was 2.6 mg/kg/minute, a finding in good agreement with previous data in dogs (49) as well as in human subjects (50, 51). The basal arterial levels of the individual amino acids are shown in Table Ia. In Figure 2 and Table II, are shown those amino acids for which a consistent exchange across the splanchnic bed was demonstrable. Noteworthy is the fact that the arterial level of alanine exceeded that of all the other amino acids with the exception of glutamine. In addition, the splanchnic uptake of alanine exceeded that of the other three major gluconeogenic amino acids by approximately four-fold. Both these findings are consistent with the observations of Felig et. al. in normal postabsorptive subjects (23, 24) and stress the important role of alanine in basal glucose homeostasis.

2. Response to Epinephrine Infusion

The infusion of epinephrine resulted in significant changes in the

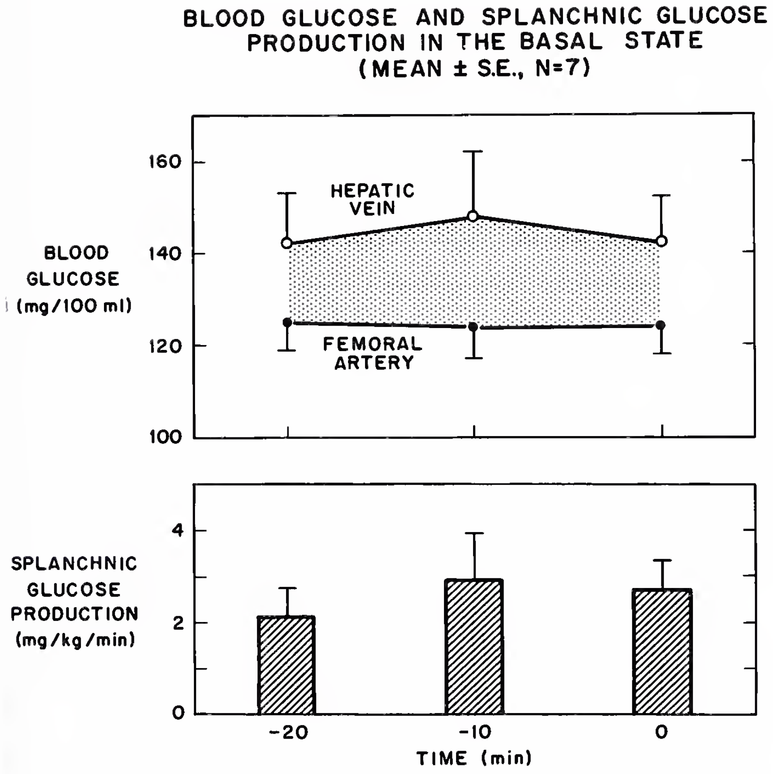

FIGURE 1 Hepatic venous and femoral arterial blood glucose, and splanchnic glucose production in seven anesthetized dogs in the basal postabsorptive state. Mean values are shown. Lines at top or bottom of circles or bars represent the standard error.

TABLE I

BLOOD GLUCOSE, SPLANCHNIC GLUCOSE PRODUCTION, AND ESTIMATED HEPATIC BLOOD FLOW IN THE BASAL STATE (N=7)

Time (min)	Blood Glucose (mg/100ml)		SGP* (mg/kg/min)	EHBF† (ml/min)
	Hepatic Vein	Femoral Art		
-20	142.5 ± 10.7Ψ	125.4 ± 5.7	2.1 ± 1.4	352 ± 56
-10	147.8 ± 14.0	123.9 ± 6.5	2.9 ± 1.0	404 ± 69
0	141.9 ± 10.1	124.4 ± 5.8	2.7 ± 1.5	407 ± 77

* Splanchnic glucose production. Calculated from the hepatic venous-femoral arterial glucose difference and the estimated hepatic blood flow.

† Estimated hepatic blood flow.

Ψ Mean ± S.E.

TABLE Ia

ARTERIAL CONCENTRATION OF 15 INDIVIDUAL AMINO ACIDS IN NORMAL POSTABSORPTIVE ANESTHETIZED DOGS

(N=4)

Amino Acid	Basal	Epinephrine*
Alanine	365 ± 72†	353 ± 51
Glycine	137 ± 15	130 ± 17
Valine	145 ± 14	145 ± 13
Proline	78 ± 8	74 ± 10
Threonine	113 ± 14	105 ± 16
Leucine	97 ± 11	101 ± 10
Serine	85 ± 8	75 ± 8
Isoleucine	53 ± 4	59 ± 2
Tyrosine	23 ± 4	21 ± 2
Taurine	46 ± 8	62 ± 18
Phenylalanine	48 ± 5	48 ± 3
Citrulline	54 ± 10	51 ± 7
Methionine	32 ± 3	31 ± 3
Glutamine	527 ± 36	450 ± 42
Glutamate	57 ± 6	53 ± 6

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* Samples were obtained 10-20 minutes after the start of the epinephrine infusion (0.1 μg/kg/min).

† Mean ± S.E., μMoles/L.

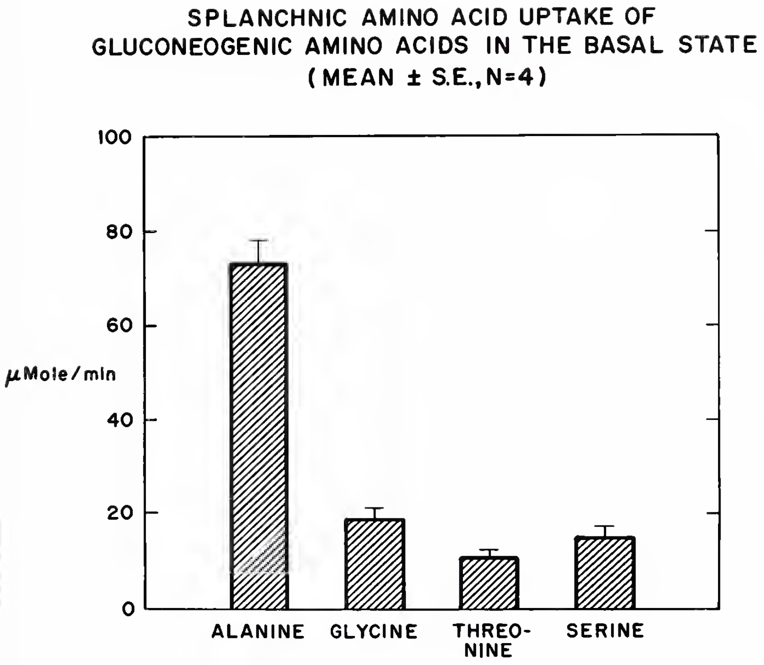

FIGURE 2 Splanchnic uptake of four gluconeogenic amino acids - alanine, glycine, threonine, and serine - in four anesthetized dogs in the basal postabsorptive state. Mean values are shown. Lines at top of bars represent the standard error.

TABLE II

SPLANCHNIC AMINO ACID UPTAKE OF FOUR GLUCONEOGENIC AMINO ACIDS IN THE BASAL STATE

(N=4)

Alanine	Glycine	Threonine	Serine
72.6 ± 5.0*	19.1 ± 2.0	11.1 ± 1.3	15.1 ± 2.2

* Mean ± S.E., μMoles/min.

arterial glucose concentration, in splanchnic glucose production, and in the uptake of alanine across the splanchnic bed. As shown in Figure 3 and Table III, the arterial glucose concentration rose significantly from a control value of 125 mg/100 ml to 144 and 142 mg/100 ml 10 to 20 minutes after beginning the infusion. At the same time splanchnic glucose production also increased approximately six-fold from a control value of 2.6 to 15.7 mg/kg/min at 20 minutes ($p<0.05$). In accord with the rise in glucose output the splanchnic uptake of three of the four gluconeogenic amino acids increased (Figure 4, Table IV). This was mainly a function of an increased splanchnic blood flow noted in the four dogs studied. The splanchnic uptake of alanine increased 37 per cent from a control value of 73 μMole/min to 100 μMole/min ($p < 0.025$). A slight increase in the splanchnic uptake of serine ($p<0.05$) and glycine ($0.1>p>0.05$) was also observed. Despite the increased splanchnic glucose production and increased hepatic uptake of alanine, arterial alanine levels did not change significantly as seen in Table Ia. Also noteworthy, was the lack of effect of epinephrine infusion on the arterial levels of the other individual amino acids (Table Ia), particularly the insulin sensitive branched-chain amino acids, phenylalanine, and tyrosine (Figure 5, Table V) (21, 52, 53). Finally, although not reaching significant levels at any one period, splanchnic blood flow tended to increase 10-20 minutes after initiation of the epinephrine infusion and remained elevated throughout the infusion and recovery periods suggesting that epinephrine probably augmented splanchnic blood flow. (Table III).

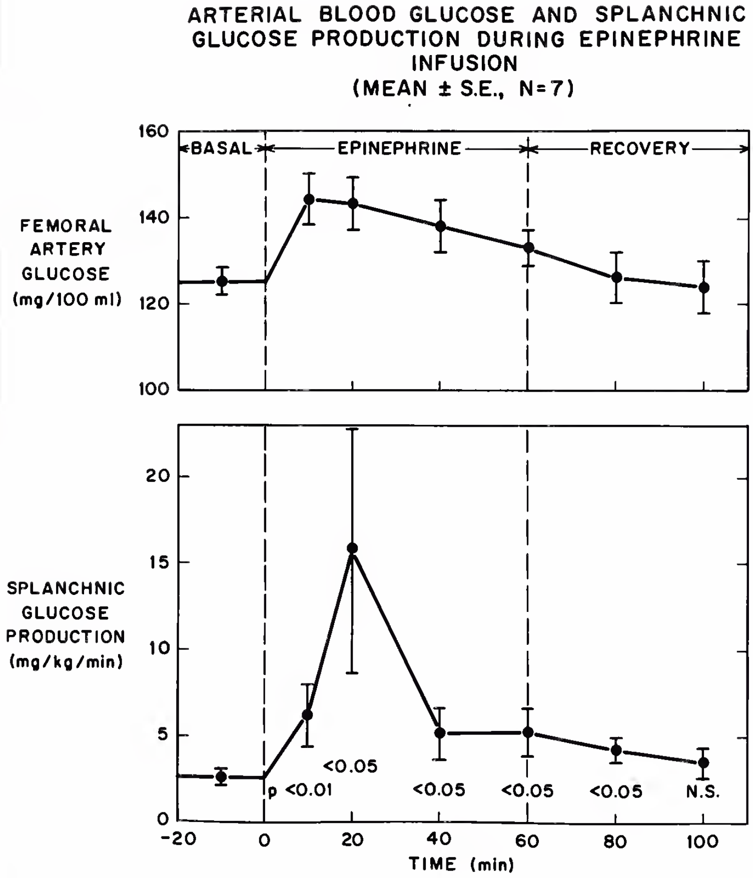

FIGURE 3 Arterial glucose levels and splanchnic glucose production in seven anesthetized dogs in the basal postabsorptive state and during and after infusion of epinephrine at 0.1 µg/kg/min. Mean values ± S.E. are shown. P values are based on paired t test.

TABLE III

ARTERIAL BLOOD GLUCOSE, SPLANCHNIC GLUCOSE PRODUCTION, AND ESTIMATED HEPATIC BLOOD FLOW DURING EPINEPHRINE INFUSION
(N=7)

	Time (min)						
	Control	+10	+20	+40	+60	+80	+100
Femoral Artery (mg/100ml)	124.6 ± 3.3*	143.6 ±6.6	143.0 ± 6.0	138.0 ± 6.4	132.9 ± 4.2	126.2 ±6.1	124.0 ± 6.2
		(<0.025)†	(<0.025)	(<0.01)	(0.1>p>0.05)	N.S.	N.S.
Splanchnic Glucose ProductionΨ (mg/kg/min)	2.6 ± 0.4	6.2 ±1.8	15.7 ± 7.1	5.1 ± 1.5	5.2 ± 1.4	4.3 ±0.7	3.5 ± 0.9
		(<0.01)	(<0.05)	(<0.05)	(<0.05)	(<0.05)	N.S.
Estimated Hepatic Blood Flow (ml/min)	389 ± 38	473 ±99	716 ± 222	622 ± 174	548 ± 132	533 ±143	446 ± 132
		N.S.	(0.1>p>0.05)	(0.1>p>0.05)	(0.1>p>0.05)	N.S.	N.S.

* Mean ± S.E.

† Probability that the number differs from the control value by chance alone (paired t test).

Ψ Calculated from the arterial-hepatic venous glucose difference and the estimated hepatic blood flow.

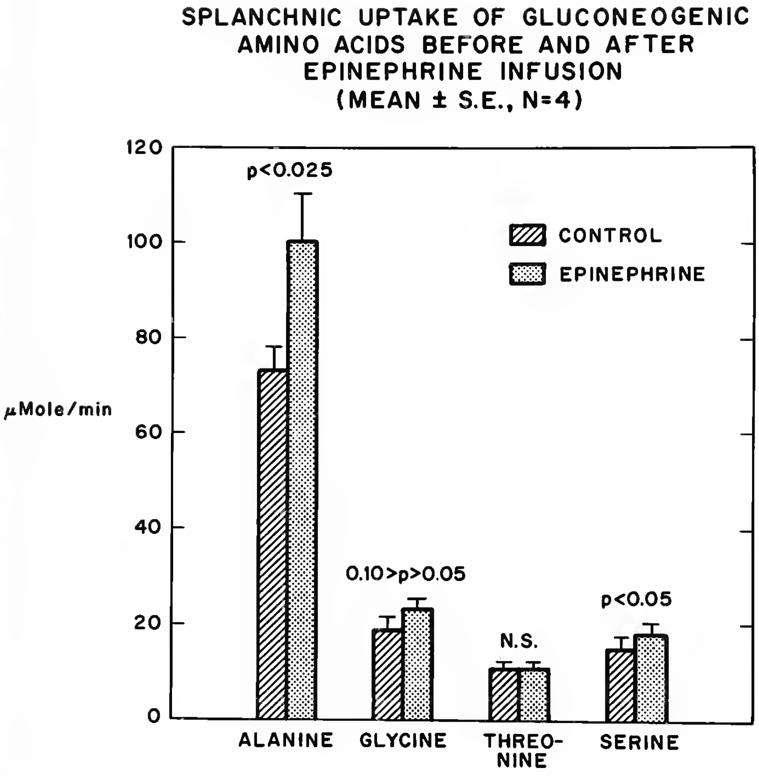

FIGURE 4 Splanchnic uptake of four gluconeogenic amino acids - alanine, glycine, threonine, and serine - in four anesthetized dogs in the basal postabsorptive state (cross-hatched bars), and 10-20 minutes after initiation of an epinephrine infusion at 0.1 μg/kg/min (stippled bars). Mean values are shown. Lines at top of bars represent the standard error. P values are based on paired t test.

TABLE IV

SPLANCHNIC UPTAKE OF FOUR GLUCONEOGENIC AMINO ACIDS BEFORE AND AFTER EPINEPHRINE INFUSION

(N=4)

	Control	Epinephrine Infusion[*]	P value[†]
Alanine	72.6 ± 5.0[Ψ]	99.8 ± 10.0	<0.025
Glycine	19.1 ± 2.0	22.7 ± 1.3	$0.1>p>0.05$
Threonine	11.1 ± 1.3	11.4 ± 0.9	N.S.
Serine	15.1 ± 2.2	18.3 ± 2.0	<0.05

[*] Samples were obtained 10-20 minutes after the start of the epinephrine infusion (0.1 μg/kg/min).

[†] P, probability that changes in splanchnic uptake after epinephrine are a chance occurrence (paired t test).

[Ψ] Mean ± S.E., μMoles/min.

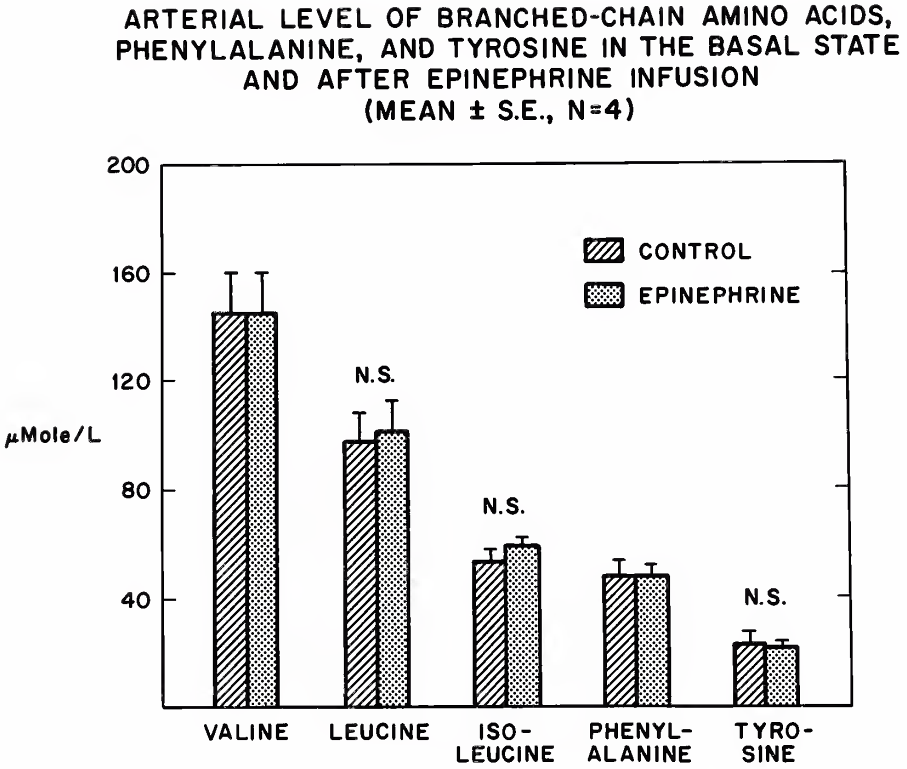

FIGURE 5 Arterial levels of the branched-chain amino acids, phenylalanine, and tyrosine in four anesthetized dogs in the basal postabsorptive state (cross-hatched bars), and 10-20 minutes after infusion of epinephrine at 0.1 μg/kg/min (stippled bars). Mean values are shown. Lines at top of bars represent the standard error. P values are based on paired t test.

TABLE V

ARTERIAL LEVEL OF THE BRANCHED-CHAIN AMINO ACIDS, PHENYLALANINE AND TYROSINE IN THE BASAL STATE AND AFTER EPINEPHRINE INFUSION (N=4)

	Control	Epinephrine*	P†
Valine	145 ± 14Ψ	145 ± 13	N.S.
Leucine	97 ± 11	101 ± 10	N.S.
Isoleucine	53 ± 4	59 ± 2	N.S.
Phenylalanine	48 ± 5	48 ± 3	N.S.
Tyrosine	23 ± 4	21 ± 2	N.S.

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* Samples were obtained 10-20 minutes after the start of the epinephrine infusion (0.1 μg/kg/min).

† P, probability that changes in arterial concentration after epinephrine are a chance occurrence (paired t test).

Ψ Mean ± S.E., μMoles/L.

II. PROPRANOLOL

1. Basal Glucose Production

As observed in the epinephrine experiment, net splanchnic release of glucose was noted during the control period. As anticipated, femoral venous glucose levels were below arterial concentrations, indicating peripheral glucose consumption in the basal state. The mean arterio-femoral venous glucose difference during this period was 5.7 mg/100 ml. Insulin levels in the basal state were 10 to 12 μU/ml (Figure 6, Table VI).

2. Response to Propranolol Infusion

The infusion of propranolol resulted in significant changes in the arterial glucose concentration, in arterial insulin levels, and in the arterio-femoral venous glucose difference. On the other hand, splanchnic glucose production showed no significant change. As shown in Figure 7 and Table VII, the arterial glucose concentration fell significantly by 10 to 15 mg/100 ml at 150 minutes. The arterial insulin concentration fell from a mean value of 11.3 μU/ml to 5.6 μU/ml at 180 minutes. Despite this fall in insulin concentrations, the arterio-femoral venous glucose difference increased three to four-fold from a basal value of 5.7 mg/100 ml to 21.6 mg/100 ml. These changes were highly significant. In marked contrast to the increase in the arterio-femoral venous glucose difference splanchnic glucose production did not change significantly from a mean control value of 3 mg/kg/min throughout the 180 minutes of observation. Particularly noteworthy is the fact that the heart rate remained above 100 beats per minute throughout the period of

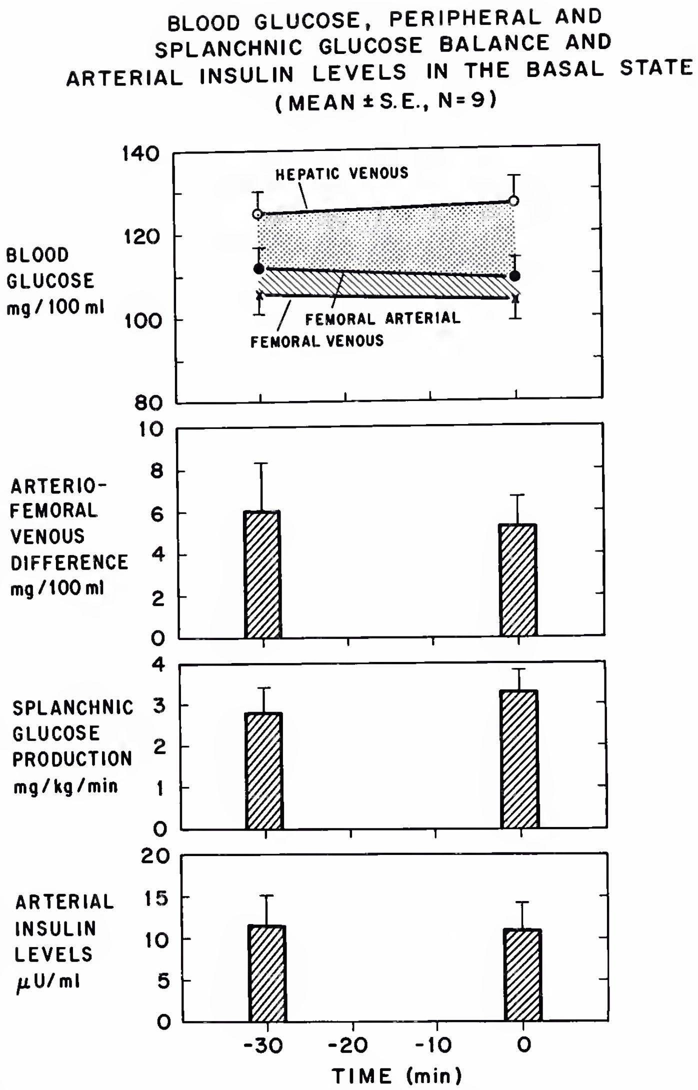

FIGURE 6 Hepatic venous, femoral arterial, and femoral venous blood glucose; arterio-femoral venous glucose difference; splanchnic glucose production; and arterial insulin levels in nine anesthetized dogs in the basal postabsorptive state. Mean values are shown. Lines at top or bottom of circles, crosses, or bars represent the standard error.

TABLE VI

BLOOD GLUCOSE, PERIPHERAL AND SPLANCHNIC GLUCOSE BALANCE, ARTERIAL INSULIN LEVELS, AND ESTIMATED HEPATIC BLOOD FLOW IN THE BASAL STATE

(N=9)

Time (min)	Blood Glucose (mg/100ml)			A-FV Glucose Difference* (mg/100ml)	SGP† (mg/kg/min)	Arterial Insulin Levels (μU/ml)	EHBFΨ (ml/min)
	Hepatic Vein	Femoral Art	Femoral Vein				
-30	124.8 ± 5.4§	111.6 ± 4.4	105.6 ± 4.8	6.0 ± 2.4	2.8 ± 0.6	11.5 ± 3.6	491 ± 106
0	126.8 ± 6.4	109.4 ± 5.3	104.1 ± 5.3	5.3 ± 1.4	3.3 ± 0.6	11.1 ± 3.0	455 ± 55

* Arterio-Femoral Venous Glucose Difference.

† Splanchnic Glucose Production. Calculated from the arterial-hepatic venous difference and the estimated hepatic blood flow.

Ψ Estimated Hepatic Blood Flow.

§ Mean ± S.E.

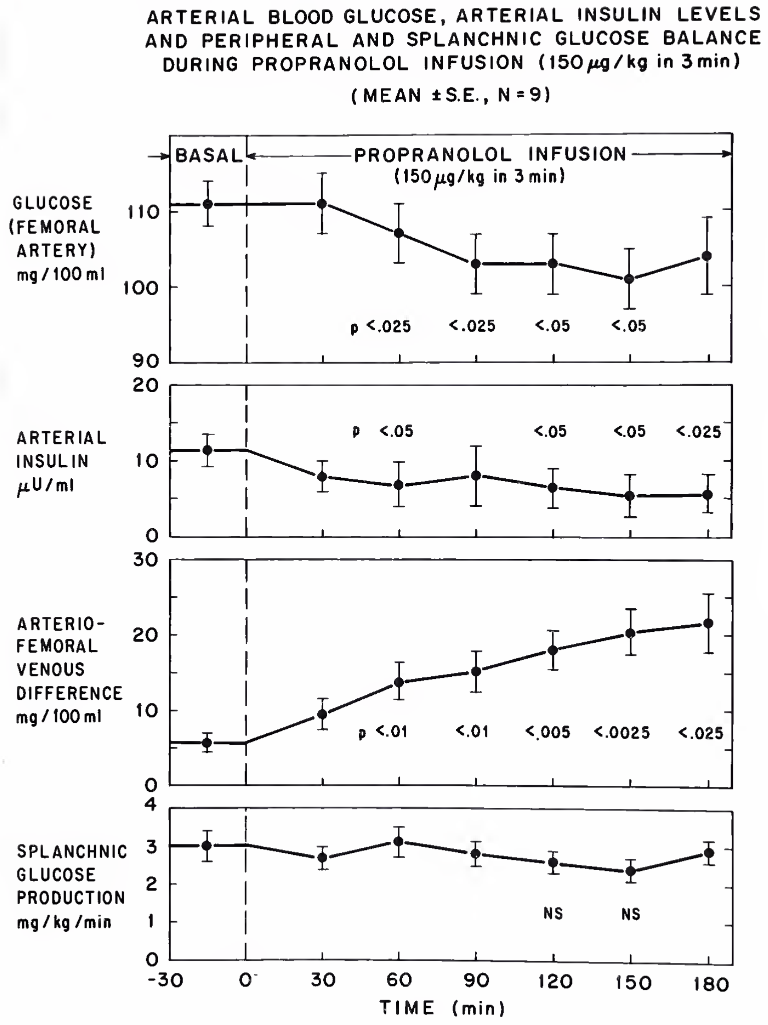

FIGURE 7 Femoral arterial glucose levels; arterial insulin levels; arterio-femoral venous glucose difference; and splanchnic glucose production in nine anesthetized dogs in the basal postabsorptive state and after a 3 minute infusion of propranolol at 150 μg/kg. Mean values ± S.E. are shown. P values are based on paired t test.

TABLE VII

ARTERIAL BLOOD GLUCOSE, ARTERIAL INSULIN LEVELS, PERIPHERAL AND SPLANCHNIC GLUCOSE BALANCE AND ESTIMATED HEPATIC BLOOD FLOW DURING PROPRANOLOL INFUSION (150 μg/kg in 3 min)

(N=9)

Time (min)	Control	+30	+60	+90	+120	+150	+180
Femoral Art (mg/100ml)	110.5 ± 3.3[*]	110.7 ± 4.1	106.9 ± 3.6	103.0 ± 3.5	102.8 ± 3.9	101.3 ± 4.2	104.2 ±5.1
		N.S.	(<0.025)[†]	(<0.025)	(<0.05)	(<0.05)	N.S.
Art Insulin (μU/ml)	11.3 ± 2.2	8.0 ± 2.1	6.9 ± 3.0	8.1 ± 4.0	6.4 ± 2.7	5.3 ± 2.8	5.6 ±2.5
		N.S.	(<0.05)	N.S.	(<0.05)	(<0.05)	(<0.025)
A-FV Glucose Difference[Ψ] (mg/100ml)	5.7 ± 1.3	9.4 ± 2.1	13.8 ± 2.5	15.1 ± 2.9	18.0 ± 2.6	20.3 ± 3.1	21.6 ±3.9
		N.S.	(<0.01)	(<0.01)	(<0.005)	(<0.0025)	(<0.0025)
SGP[§] (mg/kg/min)	3.0 ± 0.4	2.8 ± 0.3	3.1 ± 0.4	2.9 ± 0.3	2.7 ± 0.3	2.5 ± 0.3	2.9 ±0.3
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
EHBF[∫] (ml/min)	473 ± 58	364 ± 44	333 ± 39	324 ± 48	299 ± 42	283 ± 39	269 ±37
		N.S.	(<0.05)	(<0.0125)	(<0.0025)	(<0.0005)	(<0.0005)

[*] Mean ± S.E.

[†] Probability that the number differs from the control value by chance alone (paired t test).

[Ψ] Arterio-Femoral Venous Glucose Difference.

[§] Splanchnic Glucose Production. Calculated from A-HV glucose difference and [∫]estimated hepatic blood flow.

observation.

3. Response to Saline Infusion

To determine if the anesthesia or catheterization procedures were of themselves responsible for the observed changes, five additional dogs were given a saline infusion and similarly studied over a four hour period. In marked contrast to the propranolol group, as shown in Figure 8 and Table VIII, the arterio-femoral venous glucose difference did not rise significantly from a mean control value of 8.2 mg/100 ml. during the course of study. Interestingly, the splanchnic glucose production fell significantly, but this was solely a consequence of a decreased hepatic blood flow, inasmuch as the arterio-hepatic venous glucose difference was unchanged. In contrast to the significant decrease in insulin noted with propranolol arterial insulin levels remained unchanged throughout the period of observation.

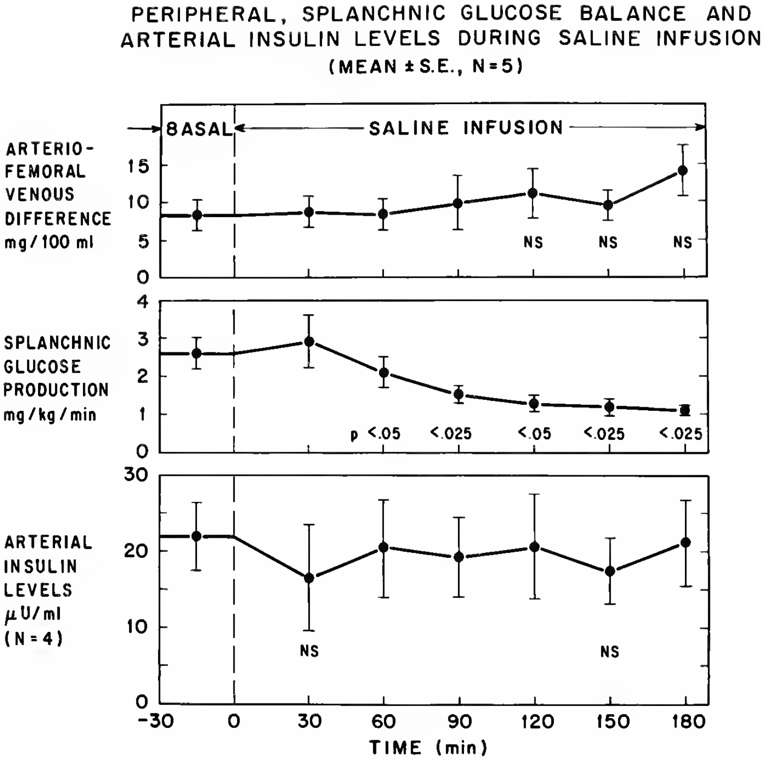

FIGURE 8 Arterio-femoral venous glucose difference; splanchnic glucose production; and arterial insulin levels in five anesthetized dogs in the basal postabsorptive state and after a 3 minute infusion of saline. Mean values ± S.E. are shown. P values are based on paired t test.

TABLE VIII

PERIPHERAL AND SPLANCHNIC GLUCOSE BALANCE, ARTERIAL INSULIN LEVELS, AND ESTIMATED HEPATIC BLOOD FLOW DURING SALINE INFUSION

(N=5)

	Time (min)						
	Control	+30	+60	+90	+120	+150	+180
Arterio-Femoral Venous Glucose Difference (mg/100ml)	8.2 ± 2.0*	8.6 ± 2.1	8.2 ± 2.1	9.8 ± 3.7	11.0 ± 3.2	9.4 ± 2.0	14.0 ±3.3
		N.S.†	N.S.	N.S.	N.S.	N.S.	N.S.
Splanchnic Glucose ProductionΨ (mg/kg/min)	2.6 ± 0.4	2.9 ± 0.7	2.1 ± 0.4	1.5 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.1 ±0.1
		N.S.	(<0.05)	(<0.025)	(<0.05)	(<0.025)	(<0.025)
Arterial Insulin Levels (μU/ml)	21.9 ± 4.5	16.5 ± 7.1	20.4 ± 6.4	19.2 ± 5.2	20.7 ± 6.8	17.5 ± 4.4	21.1 ±5.7
		N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
EHBF § (ml/min)	402 ± 40	427 ± 65	324 ± 45	236 ± 32	200 ± 38	186 ± 27	171 ±21
		N.S.	(<0.025)	(<0.0125)	(<0.0125)	(<0.005)	(<0.005)

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* Mean ± S. E.

† Probability that the number differs from control value by chance alone (paired t test)

Ψ Caluculated from A-HV glucose difference and §estimated hepatic blood flow.

DISCUSSION:

I. EPINEPHRINE

Himms-Hagen in her review of the sympathetic regulation of metabolism in 1967 (54) pointed out the large gap in knowledge with respect to the effects of epinephrine on protein and amino acid metabolism. Up to that point in time epinephrine was felt to decrease the concentration of amino acids in the blood (16, 55, 56). It was postulated that accelerated gluconeogenesis from amino acids would be a logical explanation for this observation but no direct evidence was available. In fact, evidence in the perfused liver suggested that epinephrine did not alter gluconeogenesis (57). However, London and Prenton reporting on the plasma amino acid response to insulin in man found a drop in plasma α-amino nitrogen after intravenous insulin and suggested that this drop might be related to the effect of epinephrine secretion following insulin-induced hypoglycemia (58). They postulated that the mechanism might be the promotion of the tissue uptake of amino acids by epinephrine and cited the work of Sanders and Riggs who showed that epinephrine stimulated the uptake of two non-metabolizable amino acids, 1-aminocyclopentanecarboxylic acid and α-aminoisobutyric acid (19). However, lacking in all these reports, were observations on the effect of epinephrine on individual arterial amino acids and their uptake or release by various tissues, particularly the liver.

In examining the current data one needs to evaluate first the baseline measurements of the individual arterial amino acids and their relationship

to gluconeogenesis in the basal state. In the basal anesthetized state measurement of fifteen individual amino acids by column chromatography revealed a pattern similar to that previously described in man (23). Of the individual amino acids measured alanine not only was present in a much greater concentration than the other amino acids but also accounted for a majority of the total net splanchnic amino acid consumption, suggesting a primary role of alanine in gluconeogenesis. This is in excellent agreement with the work of Felig et. al. who demonstrated that the gluconeogenic amino acids - alanine, glycine, threonine, and serine - account for over 85 per cent of the net splanchnic uptake of amino acids in normal postabsorptive subjects with alanine by far representing the largest amino acid precursor of new glucose formation (21). In fact, the basal uptake of alanine reported in this study agrees quite closely with that described in man (24, 51). The current data therefore testifies to the role of alanine in basal postabsorptive gluconeogenesis and reconfirms the concept of the glucose alanine-cycle described by Felig et. al. (22). Thus, in the present study, alanine has been shown to be the predominant arterial amino acid as well as being significantly extracted by the liver for gluconeogenesis.

The results of the epinephrine infusion suggest that in exerting its hyperglycemic effect, this hormone not only may influence glycogenolysis but also may augment gluconeogenesis. Following initiation of the infusion, an immediate rise in both arterial glucose and splanchnic glucose production was noted at 10 and 20 minutes concurring with the previous work of Bearn et. al. (8) and Porte (12). These investigators demonstrated a similar rise in plasma glucose levels and splanchnic glucose production in human subjects during a constant infusion of epinephrine at a dose

equivalent to that employed in the present study.

Concurrent with the rapid rise in arterial glucose and splanchnic glucose production, the splanchnic uptake of alanine increased by 37 per cent during the initial 10 to 20 minutes after beginning the infusion. In view of previous observations that alanine is the key endogenous substrate for gluconeogenesis (22) and its availability is the rate limiting step in gluconeogenesis (20) the current data suggests that epinephrine stimulates gluconeogenesis as well as glycogenolysis. Furthermore, inasmuch as the increase in alanine uptake was not due to a rise in arterial concentration of this amino acid, the data suggests that the stimulatory effect of epinephrine on gluconeogenesis is a consequence of a direct hepatic effect rather than a result of an action on peripheral precursor supply.

However, it was also observed that, although the absolute rate of alanine uptake increased in the experimental period as compared with the control period, the potential yield of new glucose from alanine as a percentage of the total splanchnic glucose production decreased. In other words, although more alanine was extracted by the liver during the epinephrine infusion, it represented less of the total glucose output as compared with control values. This observation suggests that in the postabsorptive state, the dog's liver is not depleted of glycogen stores and that glycogen breakdown along with glucose formation from precursors such as lactate make up the largest share of the observed increase in splanchnic glucose output after epinephrine. Support for this conclusion comes from the work done on glucose homeostasis during starvation. The data of

Felig and co-workers demonstrated similar postabsorptive values for arterial alanine levels and splanchnic uptake as in the present study (24, 51). However, after a 36 to 48 hour fast these investigators demonstrated a significant, almost two-fold increase in splanchnic alanine extraction (20), suggesting that postabsorptively the liver does not rely significantly on amino acid precursors to maintain splanchnic glucose production. It is not until at least 36 to 48 hours later when glycogen stores are presumably depleted that gluconeogenesis assumes a more important role in maintaining glucose homeostasis. This does not detract from the observation in the present study that in the postabsorptive state epinephrine is capable in small amounts of causing a significant increase in amino acid uptake, presumably for gluconeogenesis. In fact, one might then expect that epinephrine-stimulated gluconeogenesis would become a more prominent factor in maintaining splanchnic glucose production as glycogen stores were depleted in the early phase of starvation.

It was also observed in the present study that arterial alanine levels did not fall despite an increase in splanchnic uptake of this amino acid. This fact suggests that there was an increase in the production of alanine peripherally. In view of this observation, it is noteworthy that epinephrine has been demonstrated to stimulate the release of lactate and pyruvate from the periphery (4, 5, 10). Also, Felig et. al. have shown that during exercise peripheral alanine formation increases as pyruvate and lactate formation increase with arterial alanine levels rising in direct proportion to the increase in arterial pyruvate (24). Therefore, it is possible that epinephrine-induced muscle glycogenolysis

stimulated alanine release as well as lactate and pyruvate release. And, as a consequence, arterial alanine levels did not decline.

Also significant in the present study was the lack of change in the arterial concentration of the individual amino acids, particularly the branched-chain amino acids, phenylalanine, and tyrosine after epinephrine administration. This fact is noteworthy in view of the suggestion of London and Prenton in 1968 that epinephrine released as a result of insulin-induced hypoglycemia may produce a lowering of plasma amino acid levels (58). The current data shows no evidence that epinephrine has a hypoaminoacidemic effect. Therefore, the effect of insulin on amino acid levels must be a direct one. This fact has been well demonstrated by Felig et. al. who have shown the direct and exquisite sensitivity of the branched-chain amino acids, phenylalanine, and tyrosine to alterations in endogenous insulin secretion in normal human subjects (52).

Lastly, the possibility must be considered that epinephrine might act indirectly through the secretion of glucagon, a hormone known to increase gluconeogenesis (10). This has been suggested by studies demonstrating that in the in vitro perfused liver epinephrine does not increase liver glucose production, whereas glucagon does (18). Further studies employing measurement of immunoassayable glucagon will be necessary to clarify this point.

II. PROPRANOLOL

The influence of β-receptor blocking agents with regard to glucose

homeostasis must be viewed against the backdrop of the mass of information available as to the role of α and β receptors in modifying glucose, insulin, and free fatty acid concentrations in plasma. Much of this work is based on the relative potency of isoproterenol, epinephrine, and norepinephrine in stimulating either α or β receptors, with isoproterenol considered a predominantly β-receptor stimulator, epinephrine both an α and β stimulator, and norepinephrine predominantly an α-receptor stimulator. It is generally agreed that α-blocking agents block epinephrine-induced hyperglycemia in rats (59) and rabbits (60); that β-blocking agents block this hyperglycemia in rats (61) and dogs (62); and, that neither agent alone blocks this response in man (63). This evidence has led to the conclusion that receptors responsible for epinephrine-mediated hyperglycemia are α and β in the rat, β in the dog, and neither α or β alone in man (54). With regard to insulin, mediation of its secretion now seems predictable on a receptor basis with α-receptors inhibiting insulin secretion and with β-receptors enhancing insulin secretion (12). In terms of the receptor schema for lipolysis the rise of free fatty acids induced by epinephrine in man can be blocked by β but not by α-blocking agents suggesting that the β-receptor mediates lipolysis (64).

Against this background, the present results indicate that propranolol causes a modest but significant decrease in arterial glucose levels. At the same time that the arterial glucose level fell, splanchnic glucose production failed to decline significantly. Moreover, the failure of splanchnic glucose production to fall despite a significant decrease in splanchnic blood flow suggests that following propranolol administration each functional liver unit actually produced more glucose, perhaps as a consequence of the decrease

in insulin levels. This lack of inhibition by propranolol of splanchnic glucose production suggests that hepatic glycogenolysis in the dog in the basal state is not solely dependent on β-adrenergic stimulation. This is in agreement with the observations made in human subjects by Antonis et. al. that neither α or β-blockade alone abolishes epinephrine-induced hyperglycemia but that β-blockade inhibits lactate production suggesting that α-receptors are located in the liver while β-receptors are located in muscle (35).

In contrast to the findings on splanchnic glucose production the arterio-femoral venous glucose difference increased by 350 per cent. The true index of peripheral glucose utilization is the product of the peripheral blood flow and the arterio-femoral venous glucose difference. Although data is lacking in the literature on the effect of propranolol on peripheral blood flow in the normal dog in the postabsorptive state, it is unlikely that a decrease in blood flow could totally account for a three to four-fold increment in the glucose concentration gradient across the limb. This is reinforced by the lack of marked changes in hemodynamics in the present study as suggested by the fact that propranolol did not cause a bradycardia in any of the dogs and had less of an effect on splanchnic blood flow than was observed in the saline controls. Also, the observed decrease in arterial glucose concentration without a concomitant fall in splanchnic glucose production strongly suggests an increase in the peripheral utilization of glucose. The data thus would appear to support the conclusion that propranolol lowers blood glucose levels by increasing peripheral glucose consumption.

As to the physiologic mechanism by which propranolol causes an increase in the peripheral glucose uptake, it is noteworthy that a 57 per cent diminution in circulating insulin levels was observed in the present

study. The latter finding is in agreement with the receptor schema for insulin as previously described and is in accord with the data of Robertson and Porte who demonstrated a similar fall in insulin levels in human subjects given an infusion of propranolol (65). In view of the hypoinsulinemia, it would appear that propranolol increases glucose uptake by increasing the peripheral effectiveness of insulin. This conclusion is in accord with the in vitro work of Abramson and Arky who found that propranolol augmented insulin effectiveness on glucose uptake by rat hemidiaphrams (31).

As to the biochemical basis of propranolol's action on glucose uptake, Kipnis et. al. have demonstrated that epinephrine inhibits insulin-stimulated peripheral glucose uptake by causing the cellular accumulation of glucose-6-phosphate (66). The latter, in turn, inhibits hexokinase activity (67), presumably making phosphorylation rather than membrane transport the rate limiting step in glucose uptake. Thus, any blockade by propranolol of the formation of glucose-6-phosphate would prevent epinephrine antagonism of insulin-induced glucose uptake (31, 68), presumably allowing not only a greater uptake of glucose but also a lower rate of insulin secretion. Thus, although data is lacking on propranolol's effect on epinephrine-stimulated muscle glycogenolysis, this hypothesis along with the data presented point to a fertile field of endeavor on the biochemical level.

The current findings also suggest that there is a significant level of endogenous sympathetic tone in the anesthetized dog. Thus, the peripheral contra-insulin effects of this basal sympathetic tone may be

obliterated with propranolol. In addition, propranolol also serves to eliminate the β-adrenergic stimulatory component of basal insulin secretion. As a consequence, serum insulin levels decline.

Finally, the clinical relevance of these findings in the dog to man lies in the increasing uses of propranolol, and thus, the caution that must be observed so as not to precipitate attacks of hypoglycemia. Also, if indeed, a major effect in man of propranolol is to increase the peripheral sensitivity of muscle and adipose tissue to insulin, then quite possibly the physician will have another tool in his armentarium in attempting to treat the brittle diabetic. This idea is supported by the recent observations of Baker et. al. that emotionally stressed, ketotic-prone diabetic children improve considerably in terms of morbidity, i.e. ketoacidosis, while maintained on prophylactic β-blocking agents such as propranolol (69). This suggests the need for further investigation and correlation of the physiologic and biochemical mechanisms by which propranolol influences glucose homeostasis.

SUMMARY:

I. EPINEPHRINE

Seven dogs were given a constant infusion of epinephrine at a rate of 0.1 μg/kg/min. over a 60 minute period and arterial glucose levels, splanchnic glucose production, arterial levels of individual amino acids, and splanchnic uptake of the gluconeogenic amino acids were measured. Arterial glucose and splanchnic glucose production increased within 10 to 20 minutes after the start of the infusion, gradually returning to baseline in the recovery period. The splanchnic uptake of alanine increased significantly. However, the arterial levels of the individual amino acids, particularly, alanine, did not change. It is concluded that in anesthetized dogs in the postabsorptive state that epinephrine exerts its hyperglycemic action not only by glycogenolysis but also by stimulating gluconeogenesis via a direct hepatic action.

II. PROPRANOLOL

Infusion of propranolol in nine dogs at a dose of 150 μg/kg over 3 minutes resulted in a decrease in arterial glucose and arterial insulin concentrations. Despite the fall in insulin, the arterio-femoral venous glucose difference increased more than three-fold while splanchnic glucose production was not consistently changed. In the five additional dogs given a saline infusion the peripheral gradient for glucose and the arterial insulin levels remained stable during a comparable four hour period of study. It is concluded that in anesthetized dogs in the postabsorptive state propranolol

reduces arterial glucose levels by increasing the peripheral utilization of glucose rather than by affecting splanchnic glucose production. This increase in peripheral glucose utilization in the face of a declining arterial insulin level suggests an increased muscle and adipose tissue sensitivity to available insulin. Furthermore, this data suggests that there is significant endogenous sympathetic tone in the anesthetized dog in the basal state and that propranolol may obliterate the contra-insulin effects of this tone. Thus, the influence of β-adrenergic activity on glucose hemeostasis is dependent in part on changes related to the peripheral effectiveness of insulin.

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